Complete set of Pending Claims

12. A method for preparing a target protein with a C-terminal thioster, comprising:

(a) expressing a recombinant precursor protien in a host cell, the precursor protein comprising the target protein fused to an intein and optionally a binding protein domain, the intein being selected from a naturally occurring intein, an intein derivative or an intein mutant, wherein the intein is capable of thiol induced cleavage; and

(b) contacting the expressed precursor protein with a thiol reagent and inducing cleavage of the intein from the precursor protein so as to form the target protein having the C-terminal thioester.

13. The method according to claim 12, wherein the intein is selected from Sce Vma and Mxe Gyr A.

14. The method of claim 12, wherein the thiol reagent is selected from 2-mercaptoethanosulfonic acid, thiophenol, dithiothreitol, and 3-mercaptopropionic acid.

15. The method according to claim 12, wherein the precursor protein is selected from a Bst DNA polymerase I large fragment, thioredoxin and a cytotoxic protein.

16. The method according to claim 12, wherein the precursor protein is selected from a maltose binding protein and paramyosin.

17. A method for expressing a recombinant protein precursor, comprising:

inserting a nucleic acid sequence encoding a target protein into a plasmid at a multiple cloning site located upstream of and in frame with a fusion gene encoding an intein and a binding protein domain wherein the intein is selected from a naturally occurring intein, an intein derivative and an intein mutant modified intein; and

introducing the plasmid into a host cell for expressing the recombinant precursor protein.

18. The method of claim 17, wherein the binding protein encoded by the nucleic acid is a chitin binding protein.

19. The method according to claim 17, wherein the multiple cloning site contains a linker sequence.

20. The method according to claim 19, wherein the linker sequence is selected from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:4.

21. The method according to claim 17, wherein the plasmid is a pTXB plasmid.

22. A method of ligating a synthetic peptide in vitro to an inactive protein so as to restore protein activity, comprising:

(a) expressing in a host cell, the protein fused to one of an intein, an intein derivative or an intein mutant intein, wherein the intein is capable of thiol induced cleavage;

(b) inducing intein mediated cleavage of the protein by adding a thiol reagent so as to form a C-terminal thioester on the protein;

(c) preparing a synthetic peptide having an N-terminal cysteine; and

(d) ligating the inactive form of the protein to the synthetic peptide to restore protein activity.

23. The method according to claim 20, wherein the protein is cytotoxic protein.

24. the method of claim 21, wherein the cytotoxic protein is a restriction endonuclease.

25. A method of labeling a target protein, comprising:

(a) expressing a recombinant precursor protein in a host cell, the precursor protein comprising the target protein fused to an intein and a binding protein domain, the intein being selected from a naturally occurring intein, an intein derivative or an intein mutant, wherein the intein is capable of thiol induced cleavage;

(b) cleaving the precursor protein in the presence of a thiol reagent so as to form the target protein having a C-terminal thioester;

(c) preparing a synthetic peptide having a marker and an N-terminal cysteine; and

(d) ligating the target protein with the synthetic peptide for labelling the target protein.

26. The method according to claim 24, wherein the marker is selected from a fluorescent marker, a spin label, an affinity tag, and a radiolabel.

27. The method according to claim 24, wherein the peptide fragment is an antigenic determinant.

28. **(New)** A method for ligating a first target protein with a second target protein, the method comprising the steps of:

(a) expressing in a host cell, a first fusion protein comprising the first target protein fused to an intein having an N-terminal cleavage activity wherein the fusion protein is expressed from a first plasmid;

(b) contacting the fusion protein of step (a) with a thiol reagent for inducing cleavage of the intein to produce a C-terminal thioester on the first target protein; and

(c) combining in a mixture for permitting ligation, the C-terminal thioester on the first target protien and a thioester reactive N-terminal amino acid on the second target protein.

29. **(New)** The method of claim 28, wherein the thioester reactive N-terminal amino acid of step (c) is a cysteine amino acid.

30. **(New)** A method according to claim 28, wherein the C-terminal thioester of step (a) is formed in the presence of a thiol reagent.

31. **(New)** The method of claim 30, wherein the thiol reagent is 2-mercaptoethanosulfonic acid.

32. **(New)** An expressed protein having a C-terminal thioester made according to claim 12.